NOTES ON BRANCHIOBDELLA.

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So little is known concerning the American members of *Branchiobdella* that the following notes may be of interest. During the summer of 1905 a number of crayfish were taken by dredging from Lake Clear, Harrietstown, Franklin County, N. Y., in about eighteen feet of water. On all of the claws the carapace, rostrum, and even on the abdomen there were large numbers of *Branchiobdella instabilia* Moore which Professor Moore has kindly identified for me. I found associated with them a few *Branchiobdella pulcherrima* and a half dozen *Bdellodrilus illuminatus*.

All of these are usually designated as external parasites but I can see no reason for using this term. The word symbiosis or commensalism more correctly describes the relation that exists between the crayfish and *Branchiobdella*; but these two terms are used with such inconsistency to-day that we can find no accepted definition of either. It is not easy to conceive how *Branchiobdella* is of benefit to the crab. Their whitish appear ance and constant movements make the crab more conspicuous yet from their location and the fact that they are attached by the posterior end suggest the probability that they do not receive anything as food from the crab's body.

The food of these forms throws some light on their habits. A study of the digestive tube in both macerated and mounted sections revealed the presence of large numbers of unicellular algæ and diatoms. The digestive tube was full and seemed to contain nothing but small plants which would suggest that the crayfish merely served to carry the worms around enabling them the better to secure food so that it seems to me that the term symbiosis used in its original and etymological sense gives a better idea of these relations than does external parasite.

All of these symbionts are continually in motion, stretching the body out in every direction as if constantly feeling for something.

During these movements *B. instabilia* change their shape, the long, slender worm quickly becomes short and broad posteriorly. I had some small clepsine in the same aquarium and was much impressed with the great similarity of movement between the two. This peculiar leech-like movement together with the prominent posterior disk by means of which the animal adheres to the crab so simulates the general external appearance of leeches that it was doubtless one of the reasons for classifying *Branchiobdella* at first with the hirudineans.

Moore, '93, first describes these two representatives of *Branchiobdella* and reports them only from Watauga Co., North Carolina, and Delaware Co., Pa., and so far as I have been able to determine they have not been recorded as occurring farther north than Pennsylvania. The water of Lake Clear is derived almost entirely from springs, but two mountain brooks flow into it, so that the water does not warm up much during the summer. The latter part of July, when the worms were taken, the temperature of the water along the shore rarely exceeded 70° F. during the day. When the crayfish having the parasites were placed in a small aquarium the *Branchiobdella* did not live more than twelve hours although the water was taken directly from the lake and changed once during that time. I am inclined to attribute their death largely to the rise in the temperature of the water as the aquarium was placed in the boat house, which was at least 15° to 20° warmer than the water in the lake.

The animals taken all seemed to be sexually mature, as subsequent study proved, and were all about the same size. Although I have over fifty specimens of *Branchiobdella instabilia* there does not seem to be any noticeable variation in their size or appearance.

The biannulation of all but the head segment in *B. pulcherrima* and the first four just back of the head in *B. instabilia* is very suggestive of leech-like affinities in these forms, which are now generally agreed as being oligochætes. The cœlomic cavity is clearly defined and perfectly divided by dissepiments into a series of cavities. The cœlomic space is encroached upon by the enormously long muscle cells which correspond to the well-known circular and longitudinal muscles of the oligochætes.

After studying a considerable number of animals in serial sections I find but one type of muscle cell. The contractile tissue is arranged around the outside of the cell and is striated in most instances. Within the contractile region there is a granular protoplasm containing a spherical nucleus which corresponds to the ordinary nucleus found in any tissue. Voigt, '86, describes several arrangements for the contractile tissue in the European form *B. varians.*

The nervous system in both *B. instabilia* and *pulcherrima* can be easily seen in a mounted specimen and the ganglionic swellings in the latter make a very clear demonstration of their relation to somites. In section the nerve strand is seen to be clearly bilaterally symmetrical.

My chief observations are concerning the development of the sex cells and the results do not confirm all of the work done by Voigt. Voigt, '85, has given a full description of the formation of the eggs and sperms of the European *Branchiobdella varians* with 135 figures. The parts of the adult sperm as described by him are the same as those in *B. instabilia.* Concerning the development of the germ cells, however, the agreement is not so satisfactory.

The nebenkorper is unmistakably described for the nucleolus in the ovocyte on page 322. In Fig. 57 "Sind die kerne der spermatocyten noch ungeteilt ; in Zweien von Zellen ist das Nebenkörperchen noch einfach, in den anderen bereits doppelt." In the figure referred to above and in succeeding ones there can be no doubt but that the nucleolus is the body described under the caption of Nebenkörperchen.

The spermatid has two nebenkörperchen, one that has persisted, the other is developed from the thicker protoplasm near the cell wall. "Das eine davon is das Nebenkörperchen, das andere besteht, wie sich herausgestellt hat aus einer Ausammlung von dichterem Protoplasma an der Zellwand, da, wo der Schwanzfaden hervorwachst und soll in folgenden als 'Bildungskörperchen des schwanzfaden' bezeichnet werden" (page 324).

In describing the parts of the adult spermatozoön the nebenkern is derived from the nebenkörperchen, and, as already cited, this latter structure persists and becomes the tip end of the

sperm. The parts of the sperm and their derivation are summarized in the following words: "Der Samenkörper besteht also jetzt (Fig. 132): (1) aus dem nebenkern, (2) dem von einer dünnen Membran gebildeten Schlauch, (3) dem asu dem Kern hervorgegangenen konischen Teil, (4) dem Verbindungsstück, welches aus dem protplasma der Zelle anstand und aus welchem, (5) der Schwanzfaden hervorgesprosst ist," page 328.

Moore, '95, describes the morphology and histology of *Bdellodrilus* in detail, a form which was first described as *Branchiobdella*, and the agreement of *B. instabilia* is so close that a separate description is not warranted. In connection with the description of the reproductive system the following statement is made: "The first steps in the development of spermatozoa begin before the worm has nearly reached full size, and proceeds continuously; the various stages floating freely in the cœlom, in which they complete their development. In the mature worm the cavities of the fifth and sixth post-cephalic somites are filled with spermatozoa in various stages of development, while the testes proper have become much reduced and inconspicuous. The details of this process have been admirably worked out and described by Voigt; and it need only be added that what observations the writer has made are in accordance with his account" (page 519). In the same year Calkins' paper appeared on the spermatogenesis of *Lumbricus* and makes no mention of the works of Voigt.

The ovaries are located in the seventh post-cephalic somite and consist of two separate sacs, one on each side of the digestive tube. The young ova in one half of the ovary may be in a state of division while the rest of the ova are in the resting state. This division is by the usual indirect process with no evidence of astral fibers. A distinct centrosome is present at each pole of the spindle, the spindle fibers arising from within the nucleus. The chromatin assumes the characteristic spireme state preceding the formation of the equatorial plate. An early prophase stage in the division of an ovum is shown in Fig. 5. Voigt figures ova having two nucleoli and believes that this is an indication of amitotic division. My sections have been studied with this particular point in mind and no case of amitosis has been noted. In a few

instances ova containing two nucleoli (Fig. 4) were found. The nucleolus is large and when mitosis begins has undergone a transformation similar to the changes described for *Haminea* (Smallwood, '05). This would indicate that the nucleolus breaks up and does not take an active part in division. Furthermore, in some cells the two centrosomes in the prophase were observed while the nucleolus was still present, the difference in size is alone sufficient to prevent confusion ; Voigt has apparently confused them in describing the process of direct division.

As the eggs mature, they are found on the surface of the ovary and can be readily distinguished by their large size as compared with the surrounding young ova. Associated with the ovary on one side only in most instances, there is found a large mass which is apparently nutritive in character (Fig. 3). An attempt was made to detect the beginning of this modification. The membrane which surrounds the ovary is continuous and passes around the nutritive mass. This structure extends from the extreme dorsal part of the cœlome of the seventh somite to the

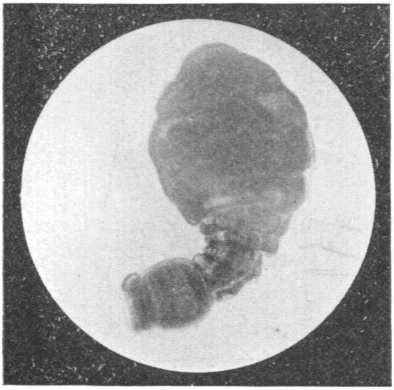

FIG. 1. A photograph of *Branchiobdella instabilia* contracted.

ventral body wall (Fig. 3). Several sections show this mass even passing beneath the intestine. Frequently the neural sheath of peritoneum can be shown to be continuous with the sac enclosing this mass and the eggs. All of the masses studied had a faint nucleus and in one instance a nucleolus was found which would

seem to indicate that this mass was a very much enlarged cell. One animal showed a few small ova in the edge of this mass adjacent to the ovary but in all other animals there was a clearly defined outline between the ova and this mass, which indicated unmistakably that the ova cells were in the ovary. This nutritive mass shows two clearly defined regions, a dorsal and ventral. The dorsal portion contains many small round bodies very uniform in size in a protoplasmic matrix. These bodies take a basic stain while Bordeaux red colors the matrix ; the ventral region does not contain these bodies, nor is it composed of granular protoplasm but yet it takes a basic stain. Its appearance in the fixed state suggests that it exists as fluid in the living animal. When the egg of the hydroid *Clava* is stained as above and viewed with the oil immersion lens, the conditions are so nearly identical that unless one knew in advance I very much doubt his ability to distinguish the two as belonging to different species. It is interesting to find this similarity in two such widely separated animals and it also helps to interpret the conditions in *Branchiobdella instabilia.* These bodies are interpreted as nutritive by Hargitt,[1] probably of a proteid nature. In *Branchiobdella*, I am inclined to believe that this nutritive mass is discharged into the cocoon where it nourishes the growing worms.

Voigt finds the beginning of what he designates a degeneration process. This process consists in the deposition of fat-bodies in certain ova but no such large size is reported nor is the change limited to one cell. Of course one can not be sure that but one cell has taken part in the formation of this large nutritive cell for the process may involve the growth of one cell at the expense of many others as is so often the case in the growth of eggs. But that this change is a fat-forming process is much to be doubted as no fat reactions were obtained. It is probably rather a normal growth process, the nutrition being stored in this special cell rather than in the cytoplasm of each egg.

What has already been said for the appearance of the ovary and manner of division may be repeated for the cells in the testes. In size, shape, reaction to stain, and appearance, they are

[1] Hargitt, C. W., "The Organization and Early Development of the Egg of Clava leptostyla," in press, BIOL. BULL.

very similar. The testes are usually somewhat larger than the ovaries and contain more cells. They are located in the sixth post-cephalic somite. Frequently the cells in the testis are pear-shaped as contrasted with the spherical form of the young ova.

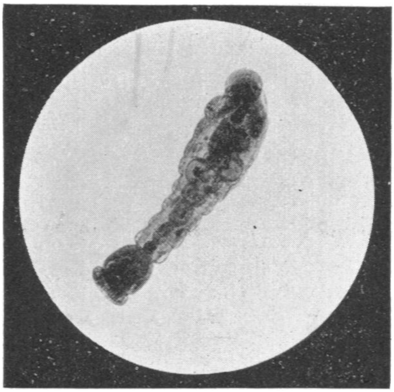

FIG. 2. A photograph of *Branchiobdella pulcherrima* showing the post-cephalic segments, the digestive tube and ganglionic swellings. In the sixth post-cephalic segment the two testes are on one side and in the seventh the ovary shows.

No evidence was observed to show that nutritive bodies such as Voigt reports for *B. varians* were present in the cytoplasm; nor is there a special nutritive cell as in the case of the ovary. In *Branchiobdella pulcherrima* the two testes were found on the same side in one animal (Fig. 2).

Associated with the formation of the male sex cells from the time they leave the testes until the spermatozoön becomes fully grown there is a protoplasmic structure, termed the blastophore (Bloomfield, '80, Calkins, '95) in *Lumbricus*, and the cytophore (Voigt, '85) in *Branchiobdella varians*. These two terms seem to refer to the same structure and the more recent term, blastophore, will be used in this paper.

Calkins traces the formation of the blastophore and finds that it appears before the sperm cells leave the testis. While still in the testis he figures the blastophore as consisting of eight nuclei in an undifferentiated protoplasm. Repeated division of the nucleus unaccompanied by the formation of cells gives rise to a multinucleate cell. After a time "cytoplasmic cleavage occurs

around each nucleus, thus differentiating the blastophore from the germ cells" (p. 275). In *B. varians* (Voigt, p. 314 sq.) the blastophore arises in a similar although not identical manner. It is first seen just as the spermatogonia escape from the testis at which time but two or four cells are associated with it. When the spermatogonia divide, a portion of the protoplasm is not enclosed within the cell wall, although it is directly connected with the cytoplasm of the cell. The young stages show the blastophore more or less irregular in shape, but as the cells become more numerous it is uniformly spherical. It differs in two particulars from the conditions in *Lumbricus*: (1) it is found in an earlier stage in the cœlome; and (2) the spermatogonia cells are always distinct in outline from the blastophore, while the earlier stage in *Lumbricus* is more like a syncytium.

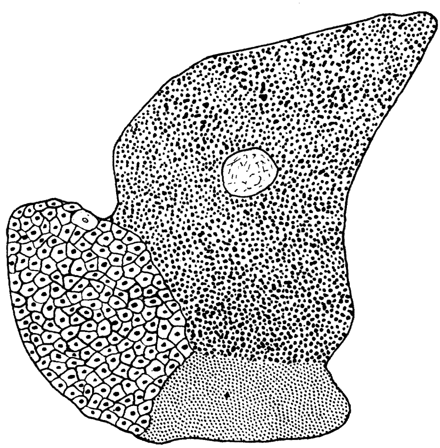

FIG. 3. A camera drawing of a single section of the ovary and nutritive mass. The dorsal granular portion contains a loosely organized nucleus. The ventral non-granular portion is larger in the other sections of it.

Branchiobdella instabilia repeats in the formation of the blastophore the conditions of *B. varians*. During the division of the spermatogonia the plane of division has a definite relation to the center of the blastophore. A definite spindle is always present with its long axis tangential to the surface of the blastophore, so that a plane passing through the equatorial plate, if continued, would go through the center of the blastophore. When the

spermatogonium divides, the division does not completely separate the two cells, but leaves them in connection with the blastophore. Each cell surrounding the blastophore has a direct protoplasmic connection with all of the other cells. Voigt finds as many as 128 cells surrounding the blastophore. One hundred cells were located with the camera lucida on the upper half of the blastophore of *Branchiobdella instabilia*, which would give about two hundred cells in all. The whole structure is so small that an accurate account is practically impossible. But from this estimate we can get some idea of the number of generations of spermatogonia and spermatocytes.

As the spermatid elongates, it remains attached to the blastophore by the tip end of the head and continues so connected until the spermatid has become a full-sized spermatozoön. The blastophore becomes reduced in size during this growth, vacuoles appear in it and the plasma stains give a more marked reaction. The reduction in size is so great in some instances that the blastophore is no larger than the leucocytes, but can be distinguished from them because of its non-nucleated condition. I believe that the blastophore is nutritive in character because of the origin and attachment of the developing spermatids and the accompanying degeneration in it as the spermatozoa approach full size (Calkins, p. 276), concludes his discussion of the blastophore with the following statement : "It lives as long as the developing germ cells are connected with it and dies when deserted by the spermatozoa. Nor is there any reason to suppose that it provides nutriment for the spermatid cell."

The phenomena of reduction are not readily determined in *Branchiobdella instabilia.* Continued search has been made to ascertain the number of chromosomes and at what stage the reduction occurred but thus far I have been unable to satisfy myself or to secure constant results. The size of the cells shown in Figs. 5 and 6 is the main reason for my failure. A study of the karyokinetic division of the cells surrounding the blastophore shows that the spindle fibers arise from the nucleus and terminate in a definite centrosome at each pole. No astral rays are present. The centrosomes lie close to the nuclear wall which gradually breaks down (Figs. 5 and 7).

As the spermatocytes become transformed into the spermatid, the chromatin gradually changes from the reticulate state into a compact, homogeneous mass. The spermatid is a small oval-shaped cell attached by the tip end of the head to the blastophore. Between the nucleus and the cell wall in the region farthest from the blastophore, there is a body which takes a basic

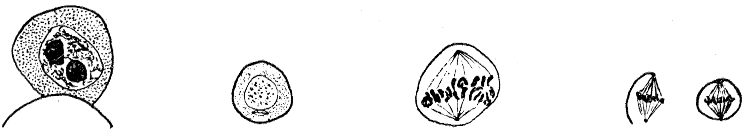

FIG. 4. A camera lucida drawing of an ovocyte with the No. 6 comp. ocular and 2 mm. objective.

FIG. 5. Prophase of young ovocyte drawn to the same magnification as Fig. 4.

FIG. 6. Metaphase of spermatocyte No. 12 comp. ocular and 2 mm. objective.

FIG. 7. Metaphase of spermatocyte showing the relation of the spindle to the nucleus, drawn to the same magnification as No. 4.

stain and is the centrosome or centrosomes of the last spermatocyte generation. The cells become so very small and the cytoplasm so much reduced that one can not be certain that there are two bodies; at this stage the body is much larger than the centrosomes during mitosis.

The distal portion of the cytoplasm becomes drawn out and forms the tail, at the same time the sperm centrosome elongates forming a connection between the nucleus and tail. This region becomes the middle piece in the adult sperm. The rapid elongation of the tail and nucleus soon renders it impossible to detect the presence of the cytoplasm. During the elongation of the nucleus, it stains more intensely and becomes constricted as if a large number of shallow parallel furrows crossed it at about 90° to the long axis of the sperm. The very tip of the sperm during nearly all of these changes remains in connection with the blastophore.

From the quotations cited from Voigt's interpretations of the spermatogenesis of the European *Branchiobdella*, it is apparent that there are important differences. In a few instances a structure which I regard as the nebenkern as described by Calkins (p. 289) was seen but this structure had no relation in its mode of origin to the nucleolus nor does it play an important rôle in

subsequent development. Voigt believes that he can trace the nebenkörpenchen from spermatogonium, through spermatocyte and spermatid to the apex of the sperm, which I very much doubt. What he has described and figured for the nebenkörpenchen in some of these early stages, I have frequently found in the free cells in the cœlome which are undoubtedly leucocytes. Kukenthal ('85, p. 454) who has made a study of the lymphoid cells of Annelids states that "The fluid of the cœlome is not homogeneous, but contains a number of elements of various kinds, among which the rather large rounded cells are the most conspicuous ; these are the lymphoid cells, and of them there are two kinds. Some have more or less finely granular protoplasm, and others contain clear highly refractive granules, which are colorless ; others are still larger, and of a yellowish-brown color." Voigt states that the nebenkörperchen can only be seen in the free living cells in some stages which is an additional confirmation of my criticism.

The statement that the spermatid has two nebenkörperchen, one of which is derived from the thickened protoplasm of the cell wall, is of course due to his inability to detect the origin of this body in the spermatocyte centrosome. The manner in which the nucleus is supposed to form the head of the sperm is somewhat unique. The head of the sperm grows out of the nucleus much as the radicle grows out of the bean, the nucleus becoming smaller as the head lengthens instead of the complete and simultaneous transformation of the whole nucleus into the head.

Branchiobdella instabilia is so closely related to *Branchiobdella varians* that the spermatogenesis of the two species is probably very nearly identical and a restudy of the latter will probably show its full agreement with the former as well as with other annelids.

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